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EVALUATING THE RELATIONSHIP BETWEEN THE SORPTION OF PAHs TO BACTERIAL BIOMASS AND BIODEGRADATION

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Abstract—Petroleum refinery wastewater containing polynuclear aromatic hydrocarbons (PAHs) are typically treated by biological processes in the United States. PAHs are recalcitrant, hydrophobic compounds and sorption to biological solids may be a significant mechanism for the removal of PAHs from refinery wastewater. The goal of this research was to investigate PAH sorption by bacterial biomass and examine the relationship between PAH biosorption and biodegradation. In this study, phenanthrene was used as a model PAH for biosorption studies and pyrene and fluoranthene were used as model compounds in biodegradation studies. It was found that phenanthrene biosorption varied with bacterial genus and species. Bacteria with the highest sorption capacity (K_p) belong to the Nocardioforms, organisms that often cause solids separation problems in activated sludge plants. Consequently, blooms of these difficult to settle organisms in refinery treatment plants could exasperate PAH releases to the environment. The measured sorption capacities were reproducible and appeared to represent surface sorption, based on the apparent competition between naphthalene and phenanthrene for sorption sites. Based on a comparison of K_p values, pure bacterial cultures can serve as valid models of biosorption by activated sludge MLSS. Finally it was found that PAH sequestration by high K_p, non-degraders has a significant impact on PAH biodegradation. The results of this study suggest that although biosorption can decrease the rate of PAH biodegradation in the short term, it can also result in the removal of PAHs from the wastewater and PAH retention in the treatment system where it may be ultimately biodegraded. This research improves our understanding of processes contributing to PAH degradation in petroleum refinery wastewater treatment plants. © 1999 Elsevier Science Ltd. All rights reserved

Key words—polynuclear aromatic hydrocarbons (PAHs), bacterial biomass, biosorption, biodegradation

INTRODUCTION

Most petroleum refinery wastewater generated in the United States is treated biologically, typically in activated sludge treatment plants. Refinery wastewater contains significant quantities of polynuclear aromatic hydrocarbons (PAHs), but relatively few studies have been published that examine the fate of PAHs in activated sludge treatment systems (Malaney, 1968; Hwang, 1981; Moretti and Neufeld, 1985, 1989; Cardinal and Stenstrom, 1991). Most studies have suggested that PAH sorption to activated sludge biosolids is an important removal mechanism (Malaney, 1968; Hwang, 1981; Moretti and Neufeld, 1985). Industry surveys of refinery biological treatment plants have suggested that sorbed PAH do not accumulate in the biosolids, which indicates that biodegradation of sorbed PAHs occurs.

Several studies have examined the relative role of sorption and biodegradation to the fate of pesticides and chlorinated aromatics in activated sludge systems (Bell and Tsezos, 1987, 1988; Weber *et al.*, 1987; Tsezos and Bell, 1988, 1989; Ettala *et al.*, 1992; Nyholm *et al.*, 1992; Jacobsen *et al.*, 1993, 1996). In most cases it was shown that sorption was a significant removal mechanism in activated sludge systems only if the compound was not biodegradable. Since many PAHs are recalcitrant, these studies with other pollutants suggest that biosorption could be a significant removal mechanism for PAHs in activated sludge treatment.

The mechanisms controlling PAH biosorption are not well understood. The sorption of PAHs to sediments and soils has been directly related to the organic carbon content of the sorbant (Karickhoff *et al.*, 1979; Dzombak and Luthy, 1984). The sorption of PAHs to bacterial cells is more complex, possibly involving both dissolution and surface active reactions. Previous studies have suggested that the sorption of pesticides to biomass may involve simultaneous surface sorption, partitioning processes and chemical reactions. The relative role of each mech-

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anism is difficult to distinguish and the predominate interaction with the biomass appeared to depend on the compound being tested (Bell and Tsezos, 1987; Tsezos and Bell, 1988).

Linear sorption isotherms are typically found in studies examining the sorption of organic pollutants to biomass. Moretti and Neufeld (1989) modeled linear PAH sorption in a study of an activated sludge system treating a coal gasification wastewater. They modeled PAH removal as a partitioning process, rather than a surface sorption phenomenon, in which PAHs partition directly into bacterial lipid bilayers. This model suggests that surface phenomena, such as competition for specific sorption sites or multilayer adsorption, would not be observed (Weber, 1972).

The physiology of the biomass itself may influence sorption processes as well. Bacteria can alter their cell surface proteins in response to their environment and the growth of bacteria under different media and growth conditions can result in changes in surface properties (Sutherland, 1988; Stringfellow *et al.*, 1991). Elucidating the relationship between cell properties and the sorption of PAHs could greatly improve our understanding of the fate of these compounds in biological treatment systems.

The objective of this research was to examine factors influencing the fate of PAHs in refinery wastewater treatment plants. Preliminary studies examining the fate of PAHs in laboratory reactors and full scale treatment plants indicated that biosorption was exerting a significant influence on the fate of PAH in refinery treatment systems. The research presented here is a systematic evaluation of the sorption of a model PAH, phenanthrene, on activated sludge and pure bacterial cultures. The cultures studied were chosen to represent organisms that might typically be found in an activated sludge treatment system (Dias and Bhat, 1964; Taber, 1976; Lemmer and Kroppenstedt, 1984). Experiments that compared PAH sorption by pure and mixed bacterial cultures were conducted to determine if pure cultures could be used to model PAH sorption by activated sludge. Relationships between physiological characteristics of the bacteria and PAH biosorption were investigated. The mechanisms of PAH biosorption and the influence of biosorption on PAH biodegradation rates were examined.

MATERIALS AND METHODS

Bacterial cultures and media

R2A agar and peptone were purchased from Difco (Detroit, MI). Mineral salts medium was made by combining 1 g KH_2PO_4, 0.86 g Na_2HPO_4, 1 g NH_4Cl, 0.06 g $MgSO_4$, 0.06 g $CaCl_2$–$2H_2O$ and 1 ml trace metal solution in 1 l of distilled–deionized water. Trace metals solution was made by combining 3.3 mg $MnSO_4$–H_2O, 6.2 mg $CuSO_4$–$5H_2O$, 7.6 mg $ZnSO_4$–$7H_2O$, 11.7 mg Na_2MoO_4–$2H_2O$ and 64.6 mg $FeSO_4$–$7H_2O$ in 1 l of 0.05 N HCl.

Bacterial cultures used in this study are listed in Table 1. Cultures were maintained on R2A slants or R2A slants supplemented with an appropriate aromatic hydrocarbon (e.g. phenanthrene for PAH degraders) at 25°C. All inoculations were made from slants to liquid media for sorption studies. *Pseudomonas aeruginosa* CV1 was isolated from refinery activated sludge based on it's ability to grow on phenol as a sole carbon source. *Gordona bronchialis* RR2 and *Rhodococcus rhodochrous* RR1 were isolated from a gasoline contaminated aquifer based on their ability to grow on toluene as a sole carbon source (Deeb and Alvarez-Cohen, 1997). *Rhodococcus erythropolis* GOMEX2 and *Pseudomonas stutzeri* P-16 are polynuclear aromatic hydrocarbon degraders (Stringfellow and Aitken, 1994, 1995; Handcock *et al.*, 1996). *Nostocodia* JS8 is a mixed culture predominated by a *Nostocodia* species responsible for bulking problems at a refinery activated sludge treatment plant (Jenkins, personal communication). These cultures were compared to other bacteria that could be expected to occur in activated sludge (Dias and Bhat, 1964; Taber, 1976; Lemmer and Kroppenstedt, 1984).

Experiments were also conducted with activated sludge biomass (mixed liquor suspended solids, MLSS) collected from both municipal and refinery activated sludge treatment systems. Samples of MLSS were kept on ice and tested within 24 h of collection. Refinery MLSS samples were taken from a full-scale plant and a laboratory scale reactor receiving the same refinery wastewater as the full-scale plant.

Sorption isotherm experiments

A modification of the fixed solids concentration procedure described by Dobbs *et al.* (1988) was used in this study to determine the equilibrium partition coefficient (K_p) for the sorption of phenanthrene by bacterial biomass. Bacterial strains selected for study were grown on 10 g/l peptone at 25°C and harvested when they reached early stationary phase. In addition, some strains were grown on phenol or naphthalene as a sole carbon source in experiments examining the influence of aromatic growth substrates on phenanthrene sorption coefficients.

Harvested cells and MLSS samples were washed and resuspended in distilled water for sorption experiments. Concentrated cells (approximately 400 mg/l final concentration) were placed in solvent washed, glass centrifuge tubes, treated with formalyn solution (37% formaldehyde) to inactivate cells and diluted to a final volume of 20 ml with distilled water. Final concentration of formaldehyde in each tube was 1.9%. It was determined in experiments using organisms that did not degrade phenanthrene that formalyn treatment did not result in a significant change in the phenanthrene sorption coefficient (K_p) between treated and untreated cells. Biomass concentration was determined by measuring absorption at 420 nm and converting to cell biomass using a coefficient of 0.29 g dry weight biomass/l per absorbance unit as determined from standard curves (Kotch, 1994).

Phenanthrene addition was achieved by adding 20 µl of 5.0, 1.0 or 0.5 mM solution in acetonitrile to each tube for a final total concentration of 5.0, 1.0 or 0.5 µM. The centrifuge tubes were sealed with Teflon lined caps and shaken at 25°C for 3 days. 3 days were allowed to achieve equilibrium of both slow and fast sorption processes as determined from initial sorption kinetic studies. Triplicate tubes were constructed for each concentration and triplicate control tubes for each concentration received no biomass. After 3 days, the tubes were centrifuged sufficiently to remove the bacteria as a pellet, the supernatant was harvested and phenanthrene in the supernatant was analyzed using HPLC with fluorescence detection. Control

Table 1. Bacterial cultures used in this study

Culture	Notable characteristic
Acinetobacter sp.	common Gram-negative
Escherichia coli W3110	common Gram-negative
Gordona bronchialis RR2	toluene degrader, nocardioform
Micrococcus luteus	common Gram-positive
Mycobacterium parafortuitum ATCC 19686	common Gram-positive, nocardioform
Nostocodia JS8 (mixed)	sludge bulking organism, nocardioform
Pseudomonas aeruginosa CV1	phenol degrader from refinery
Pseudomonas fluorescens	common Gram-negative
Pseudomonas stutzeri ATCC 11607	common Gram-negative
Pseudomonas stutzeri P-16	PAH degrader
Rhodococcus erythropolis GOMEX2	PAH degrader, nocardioform
Rhodococcus rhodochrous RR1	toluene degrader, nocardioform

tubes without biomass were compared to tubes with biomass to determine the amount of phenanthrene sorbed.

Sorption of phenanthrene by biomass was fitted to a linear sorption model given as follows:

$$K_p = (P_x/P_s)$$

Where P_x = mass of phenanthrene sorbed per gram of bacterial dry weight and P_s = mass of dissolved phenanthrene per liter solution, yielding a K_p in l/g biomass. The experimental method developed for this study yielded reproducible results that allowed the quantification of sorption coefficients (K_p) and 95% confidence intervals using a common spreadsheet program.

Biodegradation of PAHs in the presence of sorbing, non-degrading biomass

For experiments examining the influence of biosorption on biodegradation, cultures of *P. stutzeri* P-16 (a PAH degrader) and *R. rhodochrous* RR1 (non-degrader with high K_p) were grown separately on 20 g/l peptone, harvested and resuspended in mineral salts media. Three treatments were tested, degraders alone, degraders plus non-degraders and a killed control. All treatments contained 40 mg/l *P. stutzeri* P-16. The degraders plus non-degraders treatment and the killed control also contained 50 mg/l *R. rhodochrous* RR1. Killed controls were made with formaldehyde as described above. At time zero, 1.24 μM fluoranthene or 0.6 μM pyrene (final concentration) were added to each vial prior to placement in a 25°C shaker. The low carbon conditions of the media precluded significant growth over the course of the experiment and biomass measurements at the completion of the experiment confirmed that the biomass concentration did not change over the course of the experiment. Samples from each vial were taken at appropriate intervals and diluted into acetonitrile (1/1) to stop the reaction and to desorb fluoranthene and pyrene. Total fluoranthene and pyrene were measured for each time-point using HPLC.

Analytical procedures

For all analyses, bacteria were grown on peptone as described above. Protein was measured by the Bradford Assay (Daniels *et al.*, 1994) using a commercial protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as the protein standard. The assay was conducted according to the manufacturer's instructions. Analysis of total carbohydrates was conducted using the phenol reaction method of Daniels *et al.* (1994). Total carbohydrate was measured by taking 0.5 ml of sample, adding 0.5 ml of a 50 g/l phenol solution, mixing and then adding 2.5 ml of concentrated sulfuric acid and mixing rapidly. This solution is allowed to stand for 10 min and then further incubated at 25°C for 15 min. The absorbance is read at 488 nm and compared to a 10 to 100 mg/l glucose standard curve. Cell surface hydrophobicity was estimated by measuring water-cell contact angles using a NRL contact angle goniometer (Hart, Mountain Lakes, NJ). Cells were grown in peptone and filtered onto 0.2 μm polycarbonate membranes to form a uniform lawn. Measurements of contact angles were made as described by Mozes and Rouxhet (1987).

To measure surface to volume ratios, bacteria were stained with crystal violet and spread on a glass slide so that measurements of the length and width of individual cells could be made with an Olympus BH-2 microscope equipped with a Sony digital video camera attached to a C-Processing computer running SIMPLE image analysis software (C-Imaging Systems, Cranberry, PA). From length and width measurements, surface to volume ratios were calculated by modeling the bacteria as prolate spheroids as follows:

$$\text{Surface area} = 2\pi b^2 + 2\pi(ab/e)\sin^{-1}e$$

$$\text{Volume} = (4/3)\pi ab^2 \qquad e = (a^2 - b^2)^{1/2}a^{-1}$$

where a = one half the length measurement and b = one half the width (Selby, 1975).

PAH concentrations in aqueous supernatants were measured using a Waters 600E HPLC system equipped with 100 μl pump-heads and a Hewlett Packard HP1046A fluorescence detector. Column temperature was maintained for all analyses at 30°C. Phenanthrene, pyrene and fluoranthene were separated by a 80% acetonitrile/20% water mobile phase with C-18 (Supelco) or a Hypersil PAH (Keystone Scientific) columns. The lower quantitation limit for this method approximately was 0.005 μM for all three PAHs.

RESULTS AND DISCUSSION

Biosorption of phenanthrene

Biosorption of phenanthrene was examined using eleven pure cultures and five mixed cultures. In all cases, phenanthrene sorption could be described adequately by a linear model ($r^2 > 0.80$, Table 2). Other studies have also observed linear sorption of PAHs and other aromatics to biomass (Weber *et al.*, 1987; Moretti and Neufeld, 1989). The use of a linear model allows the sorption by different sources of biomass to be compared using the K_p and 95% confidence intervals (Table 2). The sorption assay was highly reproducible and the K_p value characteristic of the source of biomass.

Samples of activated sludge taken from a full scale refinery treatment plant and a pilot scale plant exhibited similar phenanthrene equilibrium sorption characteristics (Fig. 1). The sorption coefficient for

Table 2. Comparison of equilibrium phenanthrene biosorption by mixed and pure bacterial cultures

Culture	K_p (l/g biomass)	Lower 95%	Upper 95%	R^2
Gordona bronchialis RR2	36	35	38	0.99
Rhodococcus rhodochrous RR1	30	29	31	0.99
Rhodococcus erythropolis GOMEX2	15	14	16	0.99
Mycobacterium parafortuitum ATCC 19686	14	13	14	0.99
Pseudomonas fluorescens	11	9.5	12	0.95
Nostocodia JS8 (mixed)	11	7.9	13	0.83
Municipal activated sludge	9.9	9.4	11	0.99
Acinetobacter sp.	9.8	8.7	11	0.95
Refinery activated sludge (laboratory)	9.6	9.2	10	0.99
Refinery activated sludge (plant)	9.0	8.6	9.4	0.99
Escherichia coli W3110	5.3	4.4	6.3	0.89
Pseudomonas stutzeri P-16	5.7	5.2	6.2	0.98
Pseudomonas stutzeri ATCC 11607	4.9	4.7	5.2	0.99
Pseudomonas aeruginosa CV1	4.8	4.2	5.3	0.95
Micrococcus luteus	2.5	2.3	2.6	0.99

these samples were not statistically different (Table 2). Additionally, activated sludge from a municipal treatment plant had a K_p for phenanthrene that was not significantly different from that for refinery MLSS (Table 2).

The relatively high K_p values for both domestic and refinery activated sludge (approximately 9.0 l/g) confirm the importance of sorption to the fate of PAHs in activated sludge systems. The consistency in results obtained with biomass sampled from experimental and full-scale plants and the similarity of sorption characteristics between activated sludge found in domestic and refinery treatment systems, suggests that sorption phenomena of activated sludge biomass are reproducible and can be incorporated into models describing the fate of hydrophobic PAHs in activated sludge systems with a high level of confidence.

Individual bacteria were also examined for their phenanthrene sorption properties and compared to the refinery activated sludge. The Gram negative bacteria *Acinetobacter* was shown to be the best individual model of both municipal and refinery MLSS by exhibiting a K_p closest to those of

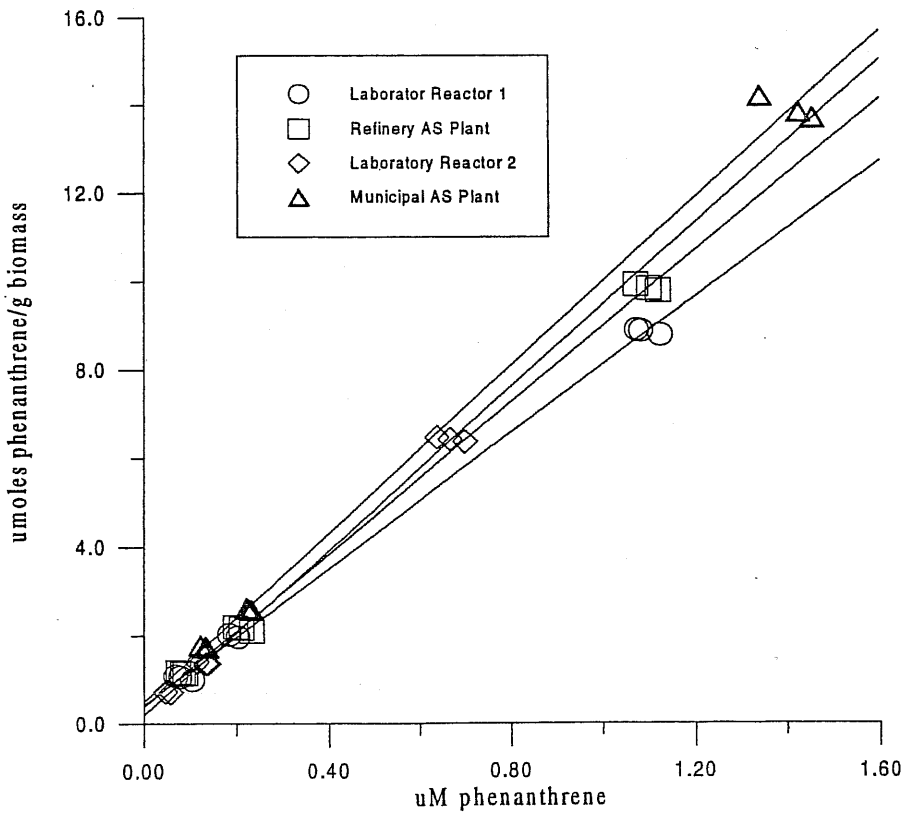


Fig. 1. Sorption of phenanthrene to biological activated sludge solids. MLSS from laboratory reactors treating refinery effluent are compared to MLSS from a full scale refinery activated sludge plant and a municipal activated sludge treatment plant.

Table 3. Factors examined for their relationship to PAH biosorption

Organism	K_p (l.g biomass)	Gram stain	Carbohydrate to protein ratio	Carbohydrate to biomass ratio	Protein to biomass ratio	Contact angle	Surface to volume ratio
Gordona bronchialis RR2	36	+	3.62	0.21	0.06	–	–
Rhodococcus rhodochrous RR1	30	+	1.98	0.12	0.06	48.3	7.3
Rhodococcus erythropolis GOMEX2	15	+	3.12	0.10	0.03	–	–
Mycobacterium parafortuitum 19686	14	+	6.12	1.16	0.19	–	12.3
Nostocodia (mixed) JS8	11	+/–	5.25	–	–	–	–
Acinetobacter sp.	9.8	–	1.21	–	–	–	–
Refinery activated sludge	9.0	+/–	0.37	–	–	–	–
Pseudomonas stutzeri P-16	5.7	–	0.55	0.95	0.89	38.2	11.3
Escherichia coli W3110	5.3	–	0.28	–	–	40.0	–
Pseudomonas stutzeri 11607	4.9	–	2.46	–	–	–	–
Micrococcus luteus	2.5	+	1.88	0.21	0.04	31.2	5.4

– = Not measured.

activated sludge samples (Table 2). Perhaps a more realistic microbial model for activated sludge MLSS would consist of a mixture of Gram positive bacteria, such as *Rhodococcus rhodochrous* and Gram negative bacteria such as *Pseudomonas stutzeri*, as MLSS is typically composed of both Gram positive and Gram negative organisms (Dias and Bhat, 1964; Taber, 1976; Lemmer and Kroppenstedt, 1984).

We tested the hypothesis that the sorption of phenanthrene would be different for bacteria that were able to degrade phenanthrene than for those that could not utilize phenanthrene. PAH degrading bacteria were compared to mono-aromatic (phenol and toluene) degraders and other activated sludge microorganisms for their ability to sorb phenanthrene. Some hydrocarbon substrates used by different organisms are listed in Table 1. Sorption coefficients were not found to be correlated with PAH degradation capabilities, nor to the abilities of bacteria to degrade hydrocarbons in general. For example, *Pseudomonas stutzeri* ATCC 11607, which will not grow on phenanthrene, naphthalene, phenol, or hexanes, exhibited a K_p similar to *Pseudomonas aeruginosa CV1*, which grows on phenol, and *Pseudomonas stutzeri P-16*, which grows on and degrades a number of PAHs.

Correlation of physiological properties with phenanthrene biosorption

In treatment systems it would be desirable to be able to measure simple chemical or physical parameters that could predict the sorptive properties of biomass. Furthermore, if the sorptive properties of activated sludge could be predicted from microbial characteristics it might be feasible to manipulate the activated sludge treatment system in such a manner as to maximize or minimize PAH biosorption as desired by the operator. To investigate this possibility, a number of physiological and chemical properties of the microbial cultures were examined to determine whether they correlated with phenanthrene biosorption (Table 3).

Carbohydrate/protein, carbohydrate/biomass and protein/biomass ratios were not correlated with phenanthrene biosorption capacity. Contact angle measurements (a higher contact angle is indicative of a more hydrophobic cell surface) were not significantly different between the different bacteria, but the higher sorbing strain tended to a higher mean contact angle. Bacterial clumping did not appear to correlate with adsorption. For example, *P. stutzeri* P-16 was characterized by clumped growth and *P. stutzeri* ATCC 11607 by dispersed growth, but both have similar K_p values.

Surface to volume ratio was also found to not reliably predict K_p. This result was somewhat surprising in light of other results presented below, but surface roughness could not be accounted for by the surface area measurement technique utilized. It is also possible that specific PAH binding sites exist and are simply more numerous on high affinity sorbers.

A positive correlation was found between carbohydrate to biomass ratios and surface to volume ratios ($r = 0.952$). This correlation is logical as bacterial carbohydrates are almost exclusively associated with the bacterial cell surface. This result supports the validity of direct measurement as a method to quantitate bacterial surface to volume ratio.

The bacteria with the highest sorption capacity were all Gram positive organisms, but the least sorbing bacterium, *Micrococcus luteus*, was also Gram positive. What seemed to be more important was that the bacteria with the highest sorption capacity belong to a group of bacteria known as Nocardioforms (Lechevalier, 1992). The high sorptive capacity of Nocardioforms is noteworthy because they are often associated with plant upset conditions that result in solids releases from activated sludge treatment systems. These results indicate that activated sludge plant effluent solids may be the source of significant PAH releases to the environment.

Table 4. Influence of growth substrate an phenanthrene biosorption coefficient (K_p)

	Growth media	K_p (l/g biomass)	Lower 95%	Upper 95%	R^2
Pseudomonas aeruginosa CV1	10 g/l peptone	4.8	4.2	5.3	0.95
Pseudomonas aeruginosa CV1	0.2 g/l phenol	5.9	5.1	6.7	0.92
Pseudomonas stutzeri P-16	10 g/l peptone	5.7	5.2	6.2	0.98
Pseudomonas stutzeri P-16	5 g/l peptone	6.5	5.7	7.2	0.96
Pseudomonas stutzeri P-16	0.025 g/l naphthalene	2.5	2.2	2.7	0.98
Pseudomonas stutzeri P-16	0.025 g/l naphthalene + 10 g/l peptone	2.8	2.1	3.4	0.85

Influence of aromatic hydrocarbon growth substrates on phenanthrene biosorption

Experiments were conducted to determine if bacterial surface properties, as measured by phenanthrene biosorption, would change when bacteria were grown on aromatic hydrocarbons typically found in refinery wastewater as compared to bacteria grown on a general media such as peptone. *P. aeruginosa* CV1, isolated from refinery activated sludge, was grown on 200 mg/l phenol and compared with the same culture grown on 10 g/l peptone. The K_p for phenanthrene sorption by *P. aeruginosa* CV1 was slightly higher when grown on phenol compared to peptone but not significantly different (Table 4).

Similar experiments comparing *P. stutzeri* P-16 grown on naphthalene to the same culture grown on peptone demonstrated that cells grown in the presence of naphthalene with or without peptone sorbed less phenanthrene than peptone grown cells (Table 4). These results suggest that PAH-degraders change their surface properties when grown on PAHs, but similar results could also be expected if naphthalene was being sorbed to PAH binding sites during cell growth and subsequently blocking phenanthrene biosorption.

Influence of co-sorbates on PAH biosorption

The influence of naphthalene on phenanthrene biosorption by *P. stutzeri* P-16 was further investigated. It was of interest to determine if the influence of naphthalene was due to physiological changes in the cell surface or competitive sorption of naphthalene on PAH binding sites.

We conducted experiments to directly test the hypothesis that PAH sorption by bacteria involves a non-specific partitioning of the PAH into bacterial cells. In the case of a partitioning reaction, at the low concentrations used in these experiments, the presence on one PAH would not be expected to influence the biosorption of other PAHs (Weber, 1972; Schwarzenbach *et al.*, 1993). However, if the hypothesis was false and PAH sorption by bacteria was due to specific surface sorption, competition between compounds might occur for surface active sites and the presence of one PAH (such as naphthalene) should reduce the sorption of other PAHs (such as phenanthrene). If multilayer adsorption occurs, the addition of one PAH could potentially enhance the sorption of another PAH (Weber, 1972). In either case, the result would be detected by a shift in the apparent sorption affinity of the bacteria for the PAH.

The surface specific or partitioning nature of PAH sorption was evaluated by examining the sorption of phenanthrene in the presence of 25 μM naphthalene as a competitive sorbate. The observed decrease in phenanthrene sorption due to the presence of naphthalene suggests that PAHs exhibit competition for surface binding sites (Fig. 2).

Effects of PAH sorption on biodegradation rates

Analysis of refinery activated sludge suggested that as little as 0.4% of the activated sludge solids were metabolically active for PAH degradation (unpublished data). This indicates that the sorption of PAH to non-degrading biomass could potentially influence the degradation of PAH in refinery wastewater treatment systems. In order to investigate the influence of non-degrading biomass on PAH degradation rates, experiments were conducted in which phenanthrene degradation rates were compared between systems containing only PAH degrading bacteria and those containing mixtures of PAH degrading and non-degrading bacteria.

A well defined PAH-degrading strain (*P. stutzeri* P-16) and a non-degrading strain (*R. rhodochrous* RR1) were selected for differences in sorptive affinity (Table 2). The cultures were grown individually as described above, washed and combined in known ratios. All treatments received the same amount of PAH degrading culture (see Section 2). The cell mixtures were tested for their ability to metabolize PAHs. The availability of sorbed fluoranthene and pyrene was reflected in the observed rates of biodegradation (Figs 3 and 4). The rate of fluoranthene degradation was reduced from 10.2 μM per g biomass per day to 3.5 μM per g biomass per day in the presence of non-degrading biomass. Pyrene degradation decreased from 4.2 μM per g biomass per day to 1.7 μM per g biomass per day. A significant reduction in the rate of both pyrene and fluoranthene degradation in the presence of non-degrading PAH biomass *in vitro* suggests that PAH sequestration by non-degraders reduces the bioavailability of PAHs to the degrading bacteria.

In full scale wastewater treatment plants operating with efficient solids separation, it is likely that biosorption will benefit PAH treatment overall by removing them from the aqueous phase and retain-

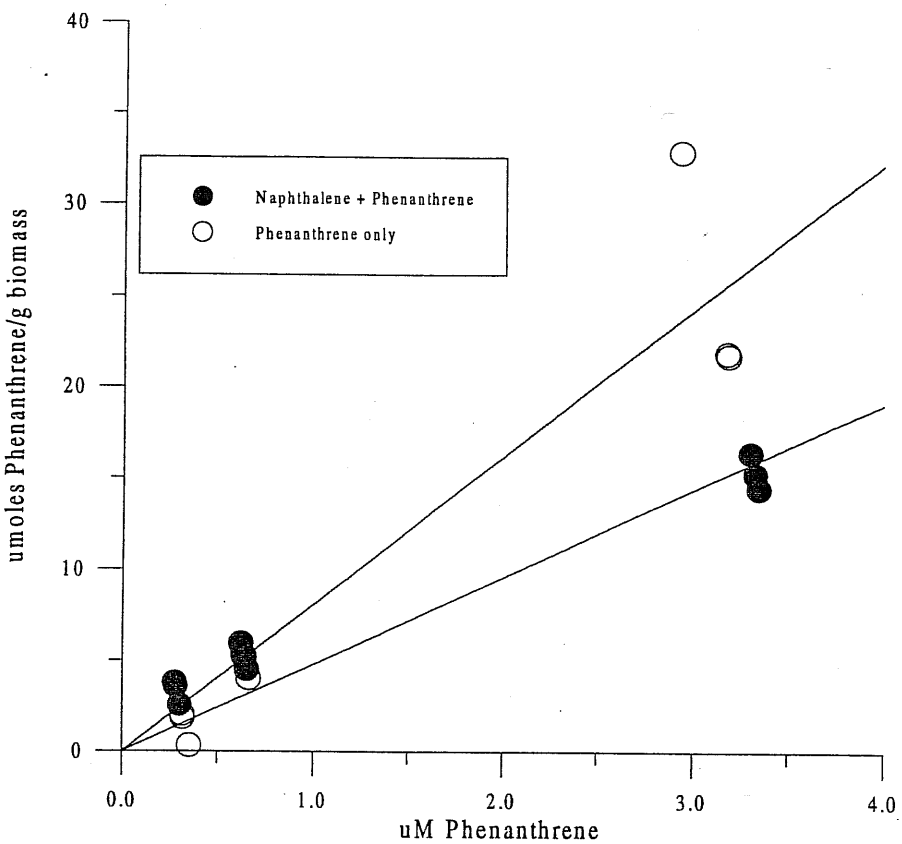


Fig. 2. The effects of 25 μM naphthalene addition as a co-sorbate on the biosorption of phenanthrene by *P. stutzeri* P-16.

ing them in the sludge solids. Sludge-associated PAHs would then be subjected to biological treatment for a period equivalent to the solids retention time rather than the hydraulic residence time. In addition, residual PAHs would be removed from the system during sludge wasting. Although the sorbed PAHs were less immediately available for biodegradation, there was no evidence in this study to suggest that sorbed PAHs would not ultimately be biodegraded.

SUMMARY AND CONCLUSIONS

The goal of this research was to provide a more complete understanding of PAH degradation by activated sludge systems treating refinery wastewater. This study specifically addressed the issue of PAH sorption by bacterial biomass and examined the relationship between PAH sorption to biomass and biodegradation. Several conclusions can be drawn from this work:

1. It was demonstrated that PAH biosorption was not predictable based upon PAH degradation capabilities or aromatic hydrocarbon degrading capabilities, but that biosorption capacity varied with bacterial species and strain.
2. The bacteria with the highest sorption capacity belong to a group of bacteria known as Nocardioforms. The high sorptive capacity of Nocardioforms is noteworthy because they are often associated with plant upsets that release solids from activated sludge treatment systems. Upset conditions that result in high effluent solids could result in significant PAH releases to the environment.
3. Sorption affinity of specific cultures were tested for correlation with gross cell characteristics such as cell hydrophobicity, cell surface area, Gram characteristic and clumping characteristics, as well as surface protein and carbohydrate content. Of these parameters, only mean cell surface hydrophobicity showed some slight positive correlation with the sorption of PAHs by bacteria.
4. Sorption capacities (K_p) can be measured reproducibly and therefore can be incorporated into models that use equilibrium values to describe the fate of hydrophobic PAHs in activated sludge systems. Our results suggest that pure bacterial cultures can serve as valid models of biosorption by activated sludge MLSS.
5. The nature of the PAH sorption was examined by measuring the sorption of an individual PAH in the presence of other PAHs as competitive sorbates. Results suggest that PAH sorption by the tested bacteria involves a surface adsorption

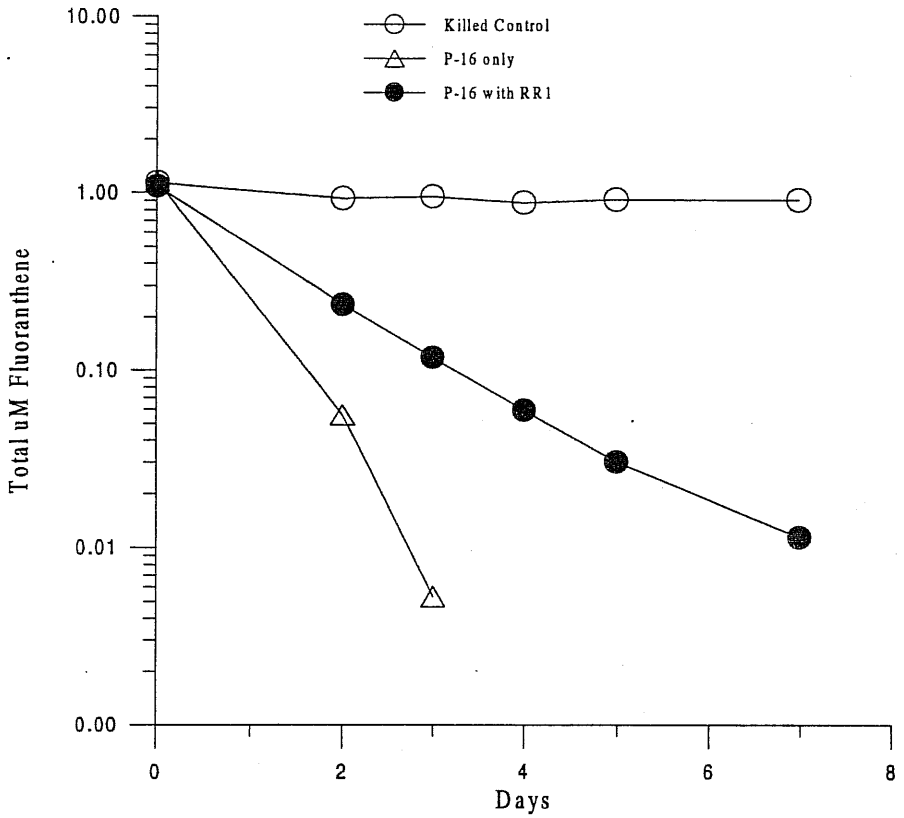
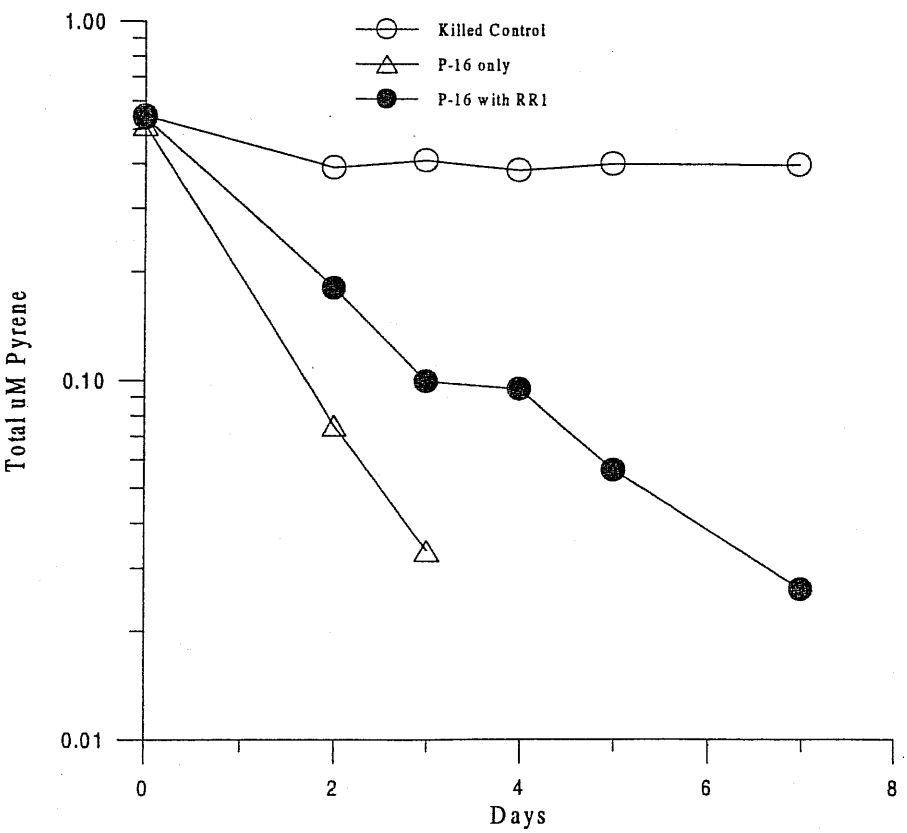


Fig. 3. The effects of a non-degrading, high sorbing biomass (*R. rhodochrous* RR1) on the rate of fluoranthene biodegradation by *P. stutzeri* P-16.



phenomenon rather than a non-specific partitioning into the bacterial cell.

6. PAH sequestration by high K_p, non-degraders had a significant impact on PAH biodegradation *in vitro*. PAH biosorption will have a significant influence on the fate of PAH in activated sludge treatment systems. The presence of high concentrations of bacteria that do not degrade PAH in activated sludge biomass may significantly limit the availability of PAHs for biodegradation in activated sludge treatment systems. Conversely, PAH biosorption may have a positive impact on PAH treatment in that sorbed PAH are retained in the treatment system for a longer period than the hydraulic residence time, so that the PAH degrading bacteria have a longer period in which to act upon the PAH.

This research improves our understanding of fundamental processes governing the degradation of PAHs in petroleum industry wastewater treatment plants. A better understanding of the relative roles of biosorption and biodegradation and the bacterial physiology of those processes, contributes to a better understanding of the fate of PAHs in biological treatment systems. Knowledge gained in this study can be used to provide insight into the improved operation of refinery activated sludge plants.

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